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LIMB SEGMENT LOAD INHIBITS THE RECOVERY OF SOLEUS H-REFLEX AFTER SEGMENTAL VIBRATION IN HUMANS

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Abstract

We investigated the effects of vertical vibration and compressive load on soleus H-reflex amplitude and post activation depression. We hypothesized that, in the presence of a compressive load, limb vibration induces a longer suppression of soleus H-reflex. Eleven healthy adults received vibratory stimulation at a fixed frequency (30 Hz) over two loading conditions (0% and 50% of individual's body weight). H-reflex amplitude was depressed ~88% in both conditions during vibration. Cyclic application of compression after cessation of the vibration caused a persistent reduction in H-reflex excitability and post-activation depression for >2.5 minutes. A combination of limb segment vibration and compression may offer a non-pharmacologic method to modulate spinal reflex excitability in people after CNS injury.

Keywords

Mechanical oscillation; compressive load; post-activation depression; spinal cord injury

INTRODUCTION

A major focus of translational regenerative research is to advance the understanding of tissue response to environmental mechanical stimuli. Mechanical oscillation (i.e. vibration) has been used to study neural and musculoskeletal adaptations in a range of people; from athletes to individuals with CNS damage from spinal cord injury (SCI) (Gilsanz et al., 2006; Iwamoto, Takeda, Sato, & Uzawa, 2005; Torvinen et al., 2003; Verschueren et al., 2004; Ward et al., 2004). Early studies have shown that vibrating directly over a muscle tendon will produce inhibitory effects on the H-reflex (Hoffmann reflex) (De Gail, Lance, &

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Neilson, 1966; Fromm & Noth, 1976; Hultborn, Meunier, Pierrot-Deseilligny, & Shindo, 1987; Lance, 1966; Lance, Degail, & Neilson, 1966; Rymer & Hasan, 1981). The inhibitory effect induced by direct tendon vibration is attributed to presynaptic inhibition, the refractory state of Ia afferent fibers, neuronal transmitter depletion at Ia terminals, postsynaptic reciprocal and non-reciprocal Ia inhibition, and effects from cutaneous and other afferent receptors (Stein, 1995).

Other studies have vibrated the whole body vertically on platforms, therefore altering gravitational load on the head (vestibular system) and body segments simultaneously. Contrary to localized muscle tendon vibration, whole body vibration induces excitatory effects on homonymous alpha motor neurons and increased synchronization of motor neuron activity (Abercromby et al., 2007; Cardinale & Bosco, 2003). Whole body vibration has been reported to have widespread effects on multiple tissues including increased muscle strength, increased bone density, and modulation of CNS plasticity (Bosco et al., 2000; Cardinale & Bosco, 2003; Gilsanz et al., 2006; Judex et al., 2005; Stein, 1995; Verschueren et al., 2004; Ward et al., 2004; Zhi, Xu, Rubin, & Hadjiargyrou, 2008). We have recently investigated a third form of vibration, in which a constrained limb segment on an oscillating platform receives a low-magnitude vibratory input. In both neurologically-intact participants and in subjects with SCI, vibration of the constrained lower limb led to alterations of H-reflex excitability and post-activation depression (Chang et al., 2012). Post-activation depression refers to the inhibition of the H-reflex after a preceding conditioning stimulus (Aymard et al., 2000; Crone & Nielsen, 1989; Hultborn et al., 1996; Kohn, Floeter, & Hallett, 1997). After SCI, reorganization of spinal circuitry leads to loss of post activation depression (Ashby, Verrier, & Lightfoot, 1974; Calancie et al., 1993; Hiersemenzel, Curt, & Dietz, 2000; Schindler-Ivens & Shields, 2000; Shields, 2002; Skinner, Houle, Reese, Berry, & Garcia-Rill, 1996). We recently showed that constrained-limb vibration training can initiate a restoration of post-activation depression, even in patients with long-standing SCI (Yen, McHenry, Petrie, Dudley-Javoroski, & Shields, 2017). Vibration training may therefore have merit as an intervention to trigger neural adaptation in patients with SCI.

In addition to vibration, compressive loads have the potential to trigger adaptive plasticity in a variety of human tissues. Axially loading a long bone and joint is believed to trigger sensory inputs that control body dynamics during standing and walking via gating of spinal circuitry (Andersen & Sinkjaer, 1999; Faist et al., 2006; Huang, Cherng, Yang, Chen, & Hwang, 2009; Knikou, Angeli, Ferreira, & Harkema, 2009a, 2009b; Petersen, Morita, & Nielsen, 1999; Phadke, Wu, Thompson, & Behrman, 2006; Trimble, Brunt, Jeon, & Kim, 2001). In patients with SCI, compressive loads induced by muscle contraction have successfully prevented loss of bone mass, loss of muscle mass, and changes in spinal reflex excitability (Adams, Suneja, Dudley-Javoroski, & Shields, 2011; Dudley-Javoroski et al., 2011; Dudley-Javoroski & Shields, 2008a, 2008b; Shields & Dudley-Javoroski, 2006; Shields, Dudley-Javoroski, & Law, 2006; Shields, Dudley-Javoroski, & Oza, 2011). We have shown that limb segment compressive load significantly decreased post activation depression of the soleus H-reflex in neurologically-intact adults, but minimally impacted excitability in individuals with chronic spinal cord injury (Tseng & Shields, 2012a). Furthermore, the magnitude of the loss of post activation depression was scaled according to the dose of load delivered to the limb segment (50% body weight (BW) > 10% BW) in healthy adults, but

was minimally modulated in those with SCI (Tseng & Shields, 2012b). Although compressive loading appears to be a powerful way to trigger adaptive plasticity for certain tissues and systems after SCI (muscle, bone), it appears to have a less-certain effect on spinal reflex plasticity. We hypothesized that an intervention combining compressive load with vibration, which elicits robust changes in spinal excitability and post-activation depression (Yen et al., 2017), could be a feasible alternative. In this study, we developed a method to synchronously deliver vibration and compressive load to a single constrained limb segment (tibia/femur joint) (Chang, Dudley-Javoroski, & Shields, 2011; Tseng & Shields, 2012a, 2012b). We applied a local mechanical vibratory input to the limb, which is in contrast to the typical whole body vibration platform studies (Iles & Pisini, 1992), and we controlled the dose of body-weight loading using a novel dynamic loading system.

The purpose of this study is to examine the effect of combining two mechanical stimuli (limb vibration and compressive load) on spinal cord reflex excitability in healthy adults. Whereas isolated limb compression had no effect on H-reflex excitability (Tseng & Shields, 2012b), we expect that a combined stimulus will reduce H-reflex excitability. We also expect a delayed, but full recovery of the soleus H-reflex following the mechanical stimulations (Chang et al., 2012).

METHODS

Subjects

Eleven healthy adults aged 21 – 30 years (mean and SD, 26.0 ± 3.7 years; seven females and four males) participated in the study. All subjects enrolled in the study have no acute or ongoing orthopedic, neuromuscular, or neurological deficits or disorders. Everyone gave informed consent before participation and the study was approved by the University of Iowa Human Subjects Institutional Review Board.

Experimental Paradigm

The study protocol was comprised of two testing sessions in order: a “vibration only” (VO) session (Figure 1A), followed by a “vibration with compressive load” (VC) session (Figure 1B). Our previous work has shown that the H-reflex for healthy adults fully returned to the baseline values after 3 min post limb segment vibration (Chang et al. 2011). Thus, each testing session was separated by 5 minutes to allow a complete washout of any effect. During the VO session, there was no compressive load applied on the lower leg segment. After the completion of the VO session, a compressive force, equivalent to 50% of the individual’s body weight, was applied over top of the left knee for 15 seconds (*load-on* phase; gray areas, Figure 1B) followed by a 15-second rest period (*load-off* phase; White areas, Figure 1B) throughout the VC session. Thus, the VC session involved fourteen 30-second cycles composed of fourteen 15-second *load-on* phases and fourteen 15-second *load-off* phases. As such, subjects experienced a constant compressive force intermittently applied on their lower leg segments throughout the entire session.

Each session consisted of three consecutive testing periods: *Baseline*, *Vibration*, and *Recovery* periods (Top panels; Figure 1A and 1B). Paired-pulse electrical stimulations

(doublets) with inter-pulse intervals of 500 ms were delivered every 15 seconds to elicit H-reflexes across all phases and sessions (Bottom panels, Figure 1A and 1B). For the VC session, each doublet was time synchronized with loading cycle. As such, the doublet was always delivered exactly in the middle of each phase in which the compressive force was continuously initiated or terminated for 7.5 seconds (dash lines, Figure 1B). During the *Baseline* period, there was no vibration while four doublets were delivered over 1 min to establish baseline H-reflexes. After the *Baseline* period, vertical oscillation stimuli were applied on the lower leg segment for 1 min (*Vibration* period), followed by a 5-min rest period (*Recovery* period). The vibration frequency was always set to 30Hz because of the known osteogenic capacity of this frequency and the amplitude of vertical oscillations on the lower leg was set to 0.6 g force. There were 4 doublets delivered during each *Vibration* period and 20 doublets delivered during the each *Recovery* period. Hence, each subject underwent two testing sessions where 56 doublets were delivered in total: 8 doublets before vibration stimuli (4 doublets in each, two *Baseline* periods), 8 doublets during vibration stimuli (4 doublets in each, two *Vibration* periods), and 40 doublets after vibration stimuli (20 doublets in each, two *Recovery* periods).

Instrumentation

Bipolar Ag-AgCl surface electrodes (8 mm in diameter, with a fixed inter-electrode distance of 20 mm) were used to record H-reflexes and M waves from the soleus muscle.

Electromyographic (EMG) signals were on-site preamplified with a gain of 35. The EMG signals were then further amplified by a CS 67 differential amplifier (Therapeutics Unlimited, Iowa City, IA) with a gain range from 1000 to 10,000. The differential amplifier had an input impedance of 15 M Ω at 100Hz, a frequency response of 15 to 1000Hz, a common mode rejection ratio of 87 dB at 60 Hz, and a bandwidth of 20 to 400 Hz.

A constant-current electrical stimulator (Digitimer Ltd, Welwyn Garden City, Herts, UK) with a range from 50 μ A to 1A and a total output capacity of 400V was used to deliver square waves with a pulse width of 1000 μ s. The stimulator was triggered by a digital pulse from a data acquisition board (USB-6259, National Instruments Corp., Austin, TX) controlled by custom LabVIEW software (National Instruments, Austin, TX). Through custom LabVIEW programming, the stimulator delivered paired-pulse electrical stimulation (doublets) with inter-pulse intervals of 500 ms every 15 seconds. Stimulation was administered via a single surface probe (cathode, 1 cm in diameter) secured over the tibial nerve in the popliteal fossa. The anode was positioned through a dispersive electrode (10 $\times$ 10 cm) placed over top of the femoral condyles.

Servo-Controlled Limb Loading

The custom designed servo-controlled system utilized an air compressor (Super Silent 20A, Silentaire Tech, Houston, TX), an electronic pressure regulator (550X Miniature E/P Transducer, ControlAir Inc, Amherst, NH), a pneumatic cylinder (Clippard USA Corp, Cincinnati, OH), a load cell (1210, Interface, Scottsdale, AZ), and custom-built integrated circuit to control the system under software control. The design allows precise control of the duration and amplitude of a compressive load over the lower leg for a pre-determined number of cycles. The compression control system generated the compressive loads in less

than 500 milliseconds. In addition, the compression control system employed a unique safety mechanism in which the pressure was continuously monitored by a pressure switch (Solon Manufacturing Co, Chardon, OH) and was allowed to shut off instantaneously when necessary. The pneumatic cylinder was positioned directly over the knee in a seated position and programmed to deliver the compressive load.

Servo-Controlled vibration

A vibration servo-control system (Ling Dynamic Systems Ltd, Royston, Herts, UK), equipped with a V721 shaker, a PA1000 linear amplifier, and a COMET controller, was used to precisely control frequency and amplitude of vertical oscillations over the lower leg. The vibration control system can generate accelerations up to 66.3g, induce continuous peak-to-peak displacement of 25.4mm, and control a range of oscillatory frequencies from zero to 400Hz with the accuracy level of 0.08dB (testing at 1000Hz sine waves). In this study, the vertical peak-to-peak displacement of the lower leg segment introduced by the vibration control system for the 0.6g acceleration condition was 0.33 millimeter.

Data Collection

Subjects sat on a custom designed chair with the hip and knee flexed at 120° and the ankle in the neutral position (Figure 2). The testing leg was secured by a Velcro strap around the foot to maintain a fixed position on the platform attached to the compressor system. Subjects were instructed to relax and sit quietly throughout the entire experiment. Subjects were also instructed to engage in light reading to maintain a similar level of consciousness throughout the entire experiment. The skin over the soleus muscle was abraded with sandpaper and cleaned with alcohol swabs. The recording EMG electrode was placed 2 cm lateral to midline of the posterior calf and 2 cm distal to the lowest end of the palpable border of the gastrocnemius muscle. A reference electrode was placed on the anterior aspect of the tibia of the right leg.

For soleus H-reflex measures, the tibial nerve was electrically activated via a stimulating electrode positioned in the popliteal fossa. The location for the stimulating electrode was determined by the conditions whereby H-reflexes were elicited without M waves at very low stimulation intensities and M waves appeared as we increased the stimulation intensity. The maximal M wave (M-max) was first defined as the peak-to-peak value at the time when the M wave amplitude failed to increase with increasing stimulation current. To ensure the tibial nerve was activated supra maximally, the stimulation intensity was then adjusted to a value greater than 120% of the last current intensity, and at least three pulses were delivered. To compare the H-reflex depression elicited by doublets across sessions and subjects, the stimulation intensity was adjusted to the level that elicited a stable soleus H-response equivalent to ~50% of the maximal H-reflex response. The H-reflexes corresponded to 15 – 25% of the M-max. All data were digitized at 4000 Hz using custom LabVIEW software and time-synchronized with the compressor and vibration systems.

Data Analysis

All analyses were conducted using Custom Matlab software (MathWorks, Natick, MA). Offline, raw EMG signals were first zero offset prior to any advanced processing. In each

doublet, paired H-reflexes 500-ms apart were first identified and were categorized as the test H-reflex (H1) and conditioned H-reflex (H2) to investigate the neural mechanism of post-activation depression (Figure 1). The peak-to-peak amplitude of each H-reflex was then computed and expressed as a percentage of M-max across three testing periods for each session. To quantify post-activation depression of soleus H-reflexes, we calculated the depression ratio by dividing the amplitude difference between the test H1 reflex and the conditioned H2 reflex by the Baseline H1 reflex average. The test H1 reflex and the depression ratios obtained during the VO and the load-on phase of VC conditions were expressed as two ratios. The normalized H1 ratio = $H1/M\text{-max}$; the normalized depression ratio = $(H1-H2)/\text{baseline mean H1}$. A depression ratio of 0 indicated that no post-activation depression occurred; whereas a ratio of 100 indicated that the greatest depression occurred such that the decrement of H2 was equal to the H1 amplitude. To quantify the modulation of the soleus H-reflex corresponding to cyclical compressive loads, H-reflex amplitudes and depression ratios were computed independently during each Load-on phase and Load-off phase within each loading cycle. Thus, we could determine whether the modulation of soleus motor neuron excitability coincides with the presence of cyclically induced loads. We did not directly compare H2 amplitudes between the VO and VC session because our previous work showed that H2 amplitude is significantly increased in the presence of compressive load (Tseng & Shields, 2012b). Therefore, the normalized depression ratios were used to account for overall changes of H1 and H2 amplitudes in response to vibration and loading. To depict H-reflex recovery over the course of time, we specifically compared normalized H1 amplitudes across three discrete time points in the *Recovery* phase to their averages during *Baseline* and *Vibration* phases: 0-min, 2.5-min, 5-min after the cessation of vibration. We chose these equally spaced time bins a priori (darker shaded condition) to reflect the changes during recovery. By using these discrete time bins we can more accurately demonstrate measurement of a dynamic condition, as we already know recovery occurs during this 5 minutes. All variables were averaged over all subjects to create group means for each session.

Statistical Analysis

Statistical comparisons were made using SAS/STAT software (SAS, Cary, NC, USA). We used a two-way (group $\times$ time) mixed model ANOVA with repeated measures on one factor (time) to test for the effects of time (baseline, vibration, 0-min, 2.5-min, and 5-min recovery) and condition (VO vs. VC). If a significant interaction was present, post hoc analyses were performed using Tukey's honest significant difference test. The level for statistical significance was set at $P < 0.05$.

RESULTS

Average H-reflex data (H1 and H2 amplitudes) for conditions, "vibration only" (VO) and "vibration compression" (VC) are shown in Figure 3A. It is evident that vibration induced an inhibitory effect on the soleus H-reflex in both VO and VC conditions (rectangles, *Vibration* periods, Figure 3A). During baseline, average H1 amplitudes were comparable between conditions (VO, 28.7% and Load-on phase of VC, 26.3% of M-max, respectively); indicating compressive load has no effect on test H1 amplitude. When vibration stimulation

was administrated, H-reflexes were suppressed in both VO and VC conditions. After vibration was terminated (Recovery period), H-reflexes for the VO condition gradually returned to the baseline level (black squares, Figure 3A) whereas recovery of test H1 amplitudes in the VC condition recovered in a distinctively different pattern. The test H1 amplitudes were suppressed during all load-on phases throughout the entire recovery period in the VC condition (gray circles in the gray areas during the Recovery period, Figure 3A). Notably, test H1 amplitudes remained low even after 2.5 min and 5 min after vibration (ovals, Figure 3A).

Similar findings of vibration-induced suppression were also observed in post activation depression ratios for both conditions (Figure 3B). Before vibration, average depression ratio was suppressed in the presence of compressive load (VO, 59.6% and Load-on phase of VC, 50.7%, respectively; Figure 3B). Due to extensive, long-lasting inhibitory effects induced by vibration, the post activation depression ratio in both conditions was close to 0 during 1-min vibration and remained at relatively lower levels compared to the baseline ratios post vibration (Figure 3B). Similar load-induced inhibitory effects on depression ratios were also observed in all load-on phases of the VC condition during the recovery period after vibration (Figure 3B). In comparison with the VO condition, depression ratios during the load-on phases were persistently smaller across the course of H-reflex recovery (ovals, Figure 3B).

By comparing over five key time points, there was a significant main effect of time ($P < 0.0001$), and a condition $\times$ time interaction effect ($P = 0.037$) on H-reflex amplitudes (Figure 4A), but no main effect of condition ($P = 0.47$). Both VO and VC conditions showed a significant decrease in H-reflex amplitude (H1) during vibration as compared to their baseline values (post hoc, $P = 0.0004$ and $P = 0.001$, respectively). For the VO condition, H-reflex amplitude at 0 min post vibration was significantly lower than the baseline value (post hoc, $P = 0.003$), but became no different from its baseline value 2.5 minutes and 5 minutes after vibration (post hoc, $P = 0.65$ and 0.97 , respectively). Surprisingly, H-reflex amplitudes for the VC condition were significantly lower than the baseline value for five minutes (post hoc, 0 min: $P = 0.02$; 2.5 min: $P = 0.04$; 5 min: $P = 0.04$; gray bars, Figure 4A); load-induced suppression of H-reflex was persistent throughout the entire recovery period of the VC condition. This finding suggests that segment loading has an inhibitory effect on H-reflex recovery after the delivery of vibration stimulation.

The depression ratio showed a significant main effect of time ($P < 0.0001$) and condition ($P = 0.042$), but no significant condition $\times$ time interaction effect ($P = 0.086$) when comparing across five key time points (Figure 4B). As expected, segmental vibration induced a significant decrease in depression ratio as compared to their baseline values for both conditions (post hoc, $P < 0.0001$) due to substantial reductions in H1 amplitudes. During the recovery phase, the depression ratios remained at lower values at 0-min and 2.5 min as compared to their baseline values (post hoc, $P < 0.0001$ and $P = 0.004$, respectively), but became no different from baseline at 5 min post-vibration (post hoc, $P = 0.89$). In addition, depression ratios during the load-on phases were persistently smaller than those during VO condition at all time points (main effect of condition, $P = 0.042$).

DISCUSSION

In this study, we investigated the effects of segmental limb vibration on spinal neuronal excitability in healthy individuals with and without compressive load. The results showed that: (1) segmental vibration resulted in H-reflex depression in both vibration-only (VO) and vibration with compression (VC) conditions, (2) prior to vibration stimuli the test H1 amplitude was stable for both unloaded and loaded conditions (baseline values for both VO and VC conditions), (3) after vibration the test H1 amplitude in the VO condition progressively returned to the baseline level whereas the test H1 amplitude in the VC condition appeared to remain at a depressed state for five minutes, and (4) Segmental vibration also induced an inhibitory effect on post activation depression up to 2.5 min post vibration in both VO and VC conditions and post activation depression ratio were persistently smaller in VC condition as compared to VO condition. The findings suggest that the combination of a mechanical compressive load combined with limb vibration may have an inhibitory effect on spinal neuronal excitability in healthy adults; likely from multiple sources including presynaptic, postsynaptic, di-synaptic, and post-activation depression mechanisms. Inhibition of spinal neuronal excitability could be of value for individuals with SCI, who develop hyperreflexia and loss of H-reflex suppression within the first 6 months post-SCI. Further work is needed to determine if routine application of a combined compression/oscillation load can prevent this deleterious reorganization of spinal circuitry after SCI.

Effects of segmental vibration on H-reflex and post activation depression

A substantial, immediate decrease in soleus H-reflex amplitude in the presence of vibration supports our first hypothesis that the segmental vibration can still effectively inhibit the H-reflex as a compressive load was cyclically applied onto the lower leg (Figure 3A). Specifically, the current study showed that, during 1-min segmental vibration, test H1 amplitudes was significantly smaller than the baseline values for both VO and VC conditions. Results of vibration-induced suppression of H-reflex are consistent with findings from our previous work, showing that directly vibrating the lower leg segment produced an inhibitory effect on soleus H-reflex (Chang et al., 2011). The test H1 amplitudes remained significantly smaller than their baseline values for 3 min in both healthy and SCI groups after the cessation of 1-min segmental vibration (Chang et al., 2011). Findings from healthy adults in this study are also consistent with findings from earlier studies demonstrating that H-reflex was suppressed to a conditioning stimulation, tendon vibration (De Gail et al., 1966; Fromm & Noth, 1976; Gillies, Lance, Neilson, & Tassinari, 1969; Hultborn et al., 1987; Lance et al., 1966; Martin, Roll, & Gauthier, 1986; Rymer & Hasan, 1981). Several underlying mechanisms are likely attributed to vibration-induced suppression of H-reflex including: 1) increased discharge threshold by group Ia afferents, 2) enhanced presynaptic inhibition at Ia terminals, and 3) transmitter depletion at Ia synapses (Curtis & Eccles, 1960; Hayward, Nielsen, Heckman, & Hutton, 1986; Hultborn et al., 1987; Stein, 1995). It is conceivable that the 1-min segmental vibration protocol from this study is analogous to the classic study paradigms of presynaptic inhibition, in which a conditioning stimulus (i.e. vibration stimulus) is delivered before the test stimulus for the H-reflex measure. Therefore, segmental vibration can be seen as a form of conditioning stimulus, similar to tendon

vibration, which enhances classic presynaptic inhibition mechanisms at Ia terminals derived from other leg muscles, including agonist and synergistic muscles around the ankle and knee. In addition to those mechanisms proposed above, we also believe that oscillating the whole lower leg synchronously vibrates antagonistic muscles (i.e. tibialis anterior), and in turn, may induce a massive post-synaptic inhibition to the soleus muscle (Martin et al., 1986). Taken together, the significant inhibition observed during and after segmental vibration was likely attributed to the sum of presynaptic and postsynaptic inhibitory mechanisms derived from continuous, homonymous activation of the muscle spindles within agonist and antagonist muscle (Martin, Roll, & Gauthier, 1984; Martin et al., 1986).

However, if one-minute of segmental vibration is analogous to a train of conditioned stimuli, homonymous synaptic depression at the Ia terminals would likely occur only during the segmental limb vibration. Thus, depression ratios were expected to stay at relatively lower values as compared to pre-vibration values for only a few seconds after termination of the segmental vibration. Indeed, we did observe that the depression ratios stayed at similar low levels for both conditions over the first two minutes in the recovery period (Figure 4B). Findings of long lasting depression of synaptic transmission from earlier studies demonstrated that depression of the soleus H-reflex was observed for more than 10 seconds following the conditioning stimulus (i.e. passive ankle dorsiflexion) (Hultborn et al., 1996). The depression ratios were significantly reduced during 1-min vibration period, suggesting synaptic efficiency of neurotransmitter release from Ia afferents was equally compromised in both conditions. The depression ratios remained smaller up to 2.5 min post vibration in both conditions and progressively returned to the baseline levels at five minutes post vibration. Hence, the post activation depression mechanism appeared to play an important role in this long-lasting vibratory-induced inhibition of the soleus H-reflex in the Recovery period (Chang et al., 2011). Individuals with acute SCI (< 6 months) demonstrate similar post-activation depression as people without SCI (Schindler-Ivens & Shields, 2000), suggesting that they, too, may demonstrate prolonged reduction of H-reflexes after vibration or vibration with compression. Clinical spasticity develops in association with the loss of normal post-activation depression after SCI (Grey et al., 2008), suggesting that an intervention that preserves post-activation depression during acute SCI could also affect the manifestation of spasticity.

Effects of compressive load on H-reflex and post activation depression

The novel findings of this study, however, were that compressive load introduced long-lasting inhibitory effects on the recovery of H-reflex amplitude (H1) when combined with a previous dose of limb segment vibration. While coupling segmental loading with vibration (i.e. VC condition), test H1 amplitudes remained significantly smaller than their baseline values throughout the entire recovery phase. This finding is supported by anecdotal reports of patients with SCI who indicate that their spasticity is depressed for minutes to hours after completing this protocol. The results did not support our second hypothesis that the recovery of H1 response is comparable between loading (i.e. VC) and unloading conditions (i.e. VO) post vibration. Before vibration, the H1 amplitudes were comparable between loading and unloading conditions, indicating that the compressive load has no effect on the test H1 response in the absence of segmental vibration. To our surprise, however, after vibration the

test H1 amplitudes were persistently suppressed to compressive load as compared to the baseline value (Figure 4A). The test H1 amplitudes in the loading condition (i.e. VC) stayed at relatively lower values at 2.5 min and 5 min post vibration whereas test H1 amplitudes in the unloading condition (i.e. VO) returned to their baseline values at 2.5 min post vibration (Figure 4A).

It is arduous to ascribe specific neuronal mechanisms to the novel findings in this study. Even though we controlled several factors by (1) applying vibration and load directly to the limb segment, and (2) synchronizing timing and duration of both stimuli delivered to the limb segment, and (3) assuring a common descending drive (EMG), we acknowledge that various sources may affect H-reflex excitability. It is possible that load-induced suppression of test H1 amplitude observed in the Recovery period was attributed to a carry-over effect from previous loading stimuli. However, we do not believe that this is the case because we used a cyclic loading protocol with a long inter-loading interval (i.e. a 15-second rest period between loads) and all tested H1 amplitudes elicited by a train of doublets could fully recover before the next cyclic load commenced. The results of test H1 amplitude across all load-off phases during the Recovery period supported this interpretation.

Findings of load-induced suppression of test H1 reflex post vibration suggested the excitability of the H-reflex pathway was altered as compared to its baseline level, likely a result of enhanced inhibition derived from multiple sources, including presynaptic, post synaptic, and disynaptic inhibitory mechanisms. It is possible that following vibration, the spinal neuronal pathways remained in a suppressed state. Adding cyclic compressive load after vibration in turn repeatedly stimulated these depressed spinal circuits and therefore augmented the overall inhibitory effects at multiple peripheral sites. A recent animal preparation verified that repeated activation of afferent pathways, particularly via Group I and II afferent inputs may be critical to reverse hyperactivity of the H-reflex after spinal cord injury (Ollivier-Lanvin, Keeler, Siegfried, Houle, & Lemay, 2010). Inhibition, via group Ib afferents, may also contribute to load-induced suppression of the H-reflex (Faist et al., 2006). Although we verified a persistent low level of background EMG at the beginning of each testing condition, we do not know the status of the inter-neuronal network in response to small changes in background EMG across the entire experimental session. It is known that supra spinal descending commands also modulate the excitatory state of the soleus alpha motor neurons via inter-neuronal pathways (Aymard et al., 2000; Bretzner & Drew, 2005). We believe that some supraspinal and spinal interneuronal pathways may be involved in H-reflex recovery post vibration (Rossi, Mazzocchio, & Schieppati, 1988). The evidence to support this position is that we showed that the rates of H-reflex recovery were different between loading and unloading conditions. Taken together, it appears that differences in H-reflex recovery between loading and unloading conditions are attributed to modulation from multiple CNS levels. Accordingly, in the absence of muscle contraction, the H-reflex excitability still relies on the interplay of the characteristics of sensory afferent inputs and the state of the inter-neuronal networks.

Findings of load-induced inhibition of post activation depression observed in this study are also consistent with our previous work as well as others (Trimble et al., 2001; Tseng & Shields, 2012a, 2012b). Specifically, limb segment load resulted in a predominant inhibition

of post activation depression up to 2.5 min after cessation of the segmental vibration. The depression ratios were persistently smaller in VC condition (i.e. loading) as compared to VO condition (i.e. unloading) post vibration.

Our previous work showed that, without segmental vibration, this load-induced reduction of post activation depression ratio resulted from a substantial increase in the conditioned H2 amplitude while the H1 amplitude remained constant (Trimble et al., 2001; Tseng & Shields, 2012a, 2012b). The stable H1 amplitude observed during the loading phase suggested that segmental compression has no effect on the excitability of the H-reflex pathway. However, a substantial increase in the conditioned H2 amplitude during loading indicated that segment compressive load suppressed the post activation depression mechanism. Indeed, our previous study in the SCI group supported this statement, demonstrating that individuals with chronic and complete SCI showed no reduction of post activation depression to segment compressive load (Tseng & Shields, 2012a). The post-activation depression ratio is known as an indicator of efficacy of monosynaptic transmission, which is measured by the depression of the H-reflex amplitude following the conditioning stimulus (Crone & Nielsen, 1989). This reduction of post-activation depression is a known phenomenon in individuals after SCI, resulting from adaptive changes of inter-neurons secondary to the loss of supra spinal control (Calancie et al., 1993; Mailis & Ashby, 1990; Schindler-Ivens & Shields, 2000). As a result of spinal re-organization, pre-synaptic inhibition at Ia afferent terminals via homonymous depression (i.e. post activation depression mechanism) is altered and are less responsive to the load (Calancie et al., 1993; Schindler-Ivens & Shields, 2000; Skinner et al., 1996; Thompson, Reier, Lucas, & Parmer, 1992). Findings from our previous research in the SCI and healthy groups suggest that multiple peripheral sensory resources including cutaneous and/or joint receptors may contribute to decreased post activation depression in response to load (Tseng & Shields, 2012a, 2012b). Previous research supports that low-threshold cutaneous afferents and joint receptors are likely activated during loading and therefore alter the excitability of spinal neuronal circuits (Conway & Knikou, 2008; Hiraoka, 2003; Iles, 1996; Knikou, 2007; Knikou & Conway, 2001; Nakazawa et al., 2004; Sayenko et al., 2007; Sayenko et al., 2009; Wood & Ferrell, 1984, 1985). Taken together, the sensory inputs from the skin and joints, generated during segment compressive load, minimally influenced the excitability of the H-reflex pathway, as supported by the constant test H1 amplitude. Furthermore, segment compressive load, via cutaneous afferents and/or joint mechanoreceptor inputs, decreased the capacity to gate afferent input via pre-synaptic inhibitory pathways and resulted in a reduction of post activation depression (Tseng & Shields, 2012a, 2012b).

Clinical Implications

After CNS lesions, H-reflex depression diminishes, reflecting a loss of post activation depression that implicates impaired inhibitory modulation within spinal neuronal circuits (Calancie et al., 1993; Mailis & Ashby, 1990; Schindler-Ivens & Shields, 2000). Clinically, a decreased ability to depress the H-reflex pathway via presynaptic inhibition is one mechanism associated with spasticity in people with SCI (Grey et al., 2008; Schindler-Ivens & Shields, 2000). While compressive loading interventions have shown promise as instigators of muscle and bone plasticity (Dudley-Javoroski et al., 2011; Shields & Dudley-

Javoroski, 2006), they have shown less utility for triggering adaptations in the monosynaptic reflex pathway (Tseng & Shields, 2012a). Recent findings from our laboratory suggest that constrained limb vibration training, an alternate form of mechanical loading, can affect post-activation depression even in individuals with long-term SCI (Yen et al., 2017). The current study demonstrated that combining vibration and compressive load can trigger long-lasting inhibitory effects on the excitability of the H-reflex pathway as well as the gating capacity at the Ia afferent terminals. This inhibition provides a framework to perhaps influence spinal cord circuitry adaptations, like collateral sprouting, which is thought to influence post activation depression loss from the acute to the chronic state after SCI (Schindler-Ivens & Shields, 2000). A provocative question is whether chronic exposure of paralyzed limbs, from the acute to the chronic state, will modulate the reorganization of the spinal circuitry to mitigate hyper-excitability of the H-reflex pathway and prevent the loss of post activation depression, which normally occur in chronic SCI (Schindler-Ivens & Shields, 2000). Future studies are necessary to understand if regularly vibrating and loading a paralyzed limb may influence the excitatory state of the alpha motor neurons, velocity dependent change in muscle stiffness (spasticity), and post activation depression of soleus H-reflex in individuals with SCI.

CONCLUSION

This study demonstrated that an intervention combining compressive and vibratory load inhibited the H-reflex and reduced post-activation depression in neurologically-intact adults. Cyclic application of compression after cessation of the vibration caused a persistent reduction in H-reflex excitability and post-activation depression for >2.5 minutes. The findings suggest that the combination of a mechanical compressive load combined with limb vibration may have an inhibitory effect on spinal neuronal excitability in healthy adults. Inhibition of spinal neuronal excitability could be of value for individuals with SCI, who demonstrate deleterious changes in H-reflex excitability within the first 6 months post-SCI. Future studies will determine the long-term effects of mechanical oscillation and compressive load on the paralyzed neuro-musculoskeletal system to develop therapeutic strategies to enhance the health of people with SCI.

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A. Vibration only

B. Vibration with compressive load

Figure 1. Illustrations of Study paradigm, vibration stimuli, cyclically compressive loads, and electrical stimulation protocols.

Two testing conditions (Vibration only, **A** and Vibration with compressive load, **B**) were assigned to each subject in order. Each condition consisted of three consecutive time periods: Baseline, Vibration, and Recovery periods, corresponding to the time duration before, during, and after vibration stimuli (upper panels, **A** and **B**). The middle panels illustrate the amplitude of timing of segmental vibration and compressive load applied to the subject's leg in each condition. The bottom traces depict soleus muscle activity and H-

reflexes elicited by doublets over time (lower panels, **A** and **B**). Arrows indicate timing when electrical stimuli were delivered to the tibial nerve. H1: test reflex; H2: conditioned reflex.

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A vibration system was attached to the lower leg segment and an air cylinder connected to a force transducer was placed over the top of the right knee joint. These systems were aligned perpendicularly to the longitudinal axis of the lower leg to deliver vertical vibration and cyclically compressive load; the force transducer and surface EMG system then recorded the net force acting on the lower leg and the soleus H-reflex response.

A. H - reflex amplitude

B. Post activation depression

Figure 3. Average H-reflexes (A) and depression ratios (B) before, during, and after vibration with/without cyclically compressive loads.

Rectangles indicate the time period when vibration stimuli were applied on the lower leg segment. Gray areas illustrate the timing and duration of cyclic compressive load for the vibration with compressive load (VC) condition. Dashed vertical lines delineate one-minute intervals after the cessation of vibration stimuli. Ovals indicate the key time points (0 min, 2.5 min, and 5 min after vibration) identified during the Recovery period for statistical comparisons. VO: vibration only; H1: test H-reflex; H2: conditioned H-reflex. Error bars, ± 1 SEM.

Figure 4. Averages of normalized H-reflexes (A) and depression ratios (B) across five key time points.

Five key time points were: before vibration (Baseline), during 1-min vibration (Vibration), and 0 min, 2.5 min, and 5 min after vibration (Recovery). Crosses (+) indicate significant post-hoc differences between a specific time point and its baseline value. Asterisks (*) indicate a significant main effect of time between a specific time point and their baseline values for both VO and VC conditions. VO: vibration only; VC: vibration with compressive load; Error bars, ± 1 SEM.